A Comprehensive Analysis Reveals Mutational Spectra and Common Alleles in Chinese Patients with Oculocutaneous Albinism

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Oculocutaneous albinism (OCA) is a heterogeneous recessive disorder with hypopigmentation in the skin, hair, and eyes. At least 16 genes have been identified as causative genes for human OCA. No comprehensive analysis has been conducted to study the spectral distribution of OCA in Chinese patients. We screened 127 unrelated and unselected Chinese OCA patients for mutations in the *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, and *HPS1* genes. We found that the spectrum of mutational genes and alleles of OCA is population specific. OCA1 is the most common (70.1% of cases) form of Chinese OCA, whereas OCA2, OCA4, and HPS1 account for 10.2%, 12.6%, and 1.6%, respectively. No apparent pathological mutation of *TYRP1* has been found. Thirty-eight previously unreported mutational alleles were identified from these OCA patients and were not found in 100 nonalbinism subjects. Of the *TYR* mutational alleles, 81.1% were clustered on exons 1 and 2. Ten common alleles account for 74.6% of the mutational *TYR* alleles in Chinese OCA1 patients. The p.D160H allele accounts for 55.6% of the mutational *SLC45A2* alleles in Chinese OCA4 patients. These results provide useful information for the establishment of an optimized strategy of gene diagnosis and genetic counseling of Chinese OCA patients.

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INTRODUCTION

Oculocutaneous albinism (OCA) is regarded as an inherited trait, with a reduction or complete absence of melanin in the skin, hair, and eyes. It is often accompanied by eye problems, including photophobia, strabismus, moderate to severe impairment of visual acuity, and nystagmus. OCA is a heterogeneous and autosomal recessive disorder. At least 16 genes have been identified as causative for OCA in humans (Tomita and Suzuki, 2004; Li *et al.*, 2006). These include four types of nonsyndromic OCA genes (*TYR/OCA1*, *P/OCA2*, *TYRP1/OCA3*, and *MATP/SLC45A2/OCA4*) and 12 syndromic OCA genes (*HPS1*, *AP3B1/HPS2*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *DTNBP1/HPS7*, *HPS8*, *LYST/CHS1*, *MYO5A/GS1*, *RAB27A/GS2*, and *MLPH/GS3*). Although *RAB38*, *SILV*, *and SLC24A5* are suggestive of candidate OCA genes, no pathological mutations have been reported in human OCA patients (Suzuki *et al.*, 2003; Hutton and Spritz, 2008a; Grønskov *et al.*, 2009). More candidate OCA genes in humans are likely to be identified as implicated by additional mouse OCA genes (Bennett and Lamoreux, 2003; Li *et al.*, 2004). Patients with autosomal recessive ocular albinism, which has been ruled out as stemming from mutations in the *GPR143/OA1* gene, have been found with mutations in the *TYR* or *OCA2* genes, which are considered clinically mild variants of OCA1 and OCA2 (Hutton and Spritz, 2008b).

The prevalence of OCA subtypes differs widely among different populations. Among African and African-American OCA patients, OCA2 is the most frequent form (King *et al.*, 2001).OCA1 has been reported to be the most frequent type of OCA in Japanese (Suzuki and Tomita, 2008), non-Hispanic Caucasians (Hutton and Spritz, 2008a), Danes (Grønskov *et al.*, 2009), and a mixed population of Europeans, Asians, and Africans (Rooryck *et al.*, 2008). Similarly, the mutational spectrum of each OCA gene varies in different populations (Suzuki and Tomita, 2008; Hutton and Spritz, 2008a; Grønskov *et al.*, 2009). In the Chinese Han population of Shangdong Province, the prevalence of OCA was reported to be 1:18,000. It is estimated that at least eight genes contribute to recessive OCA, and 3.83% of the general population of Shangdong Province are carriers (Gong *et al.*, 1994). Thus, a

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Abbreviations: HPS, Hermansky–Pudlak syndrome; OCA, oculocutaneous albinism; SNP, single-nucleotide polymorphism

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large number of individuals with OCA would be expected to be born annually in such a huge population (1.3 billion). Mutational screenings have been conducted in a small number of Chinese OCA patients, and several previously unreported mutational alleles of *TYR*, *OCA2*, and *SLC45A2* have been identified in the Chinese population (Li *et al.*, 2006, 2008; Wang *et al.*, 2009a). However, no comprehensive molecular analysis has been conducted to reveal the spectral distribution of Chinese OCA. In this study, 127 OCA patients from the Chinese Han population, the largest population in China, were recruited for extensive mutational screening of *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, and *HPS1*.

RESULTS

OCA1 is the most common type of OCA in Chinese patients

Of the 127 OCA patients, 74 were clinically diagnosed with OCA1, 35 were diagnosed with OCA2, and 18 were not differentially diagnosed because of unclear onset history (Table 1).Of these patients, 94.5% (120 of 127) were confirmed by molecular testing, with the remaining 5.5% (7 of 127) unidentified after mutational screening of the *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, and *HPS1* genes. Of the 120 molecularly diagnosed patients, 99 carried two mutational alleles and 21 carried one mutational allele. Of the identified patients, we found apparent pathological *TYR* mutations in 70.1% of the patients (89 of 127), *OCA2* mutations in 10.2% (13 of 127), *SLC45A2* mutations in 12.6% (16 of 127), and *HPS1* mutations in 1.6% (2 of 127) (Table 1). No mutation in *TYRP1* was found in these patients. Hence, OCA1 is the predominant type of OCA in the Chinese Han population.

Allelic frequencies of *TYR* in Chinese OCA patients

Of the 89 OCA1 patients, 80 had two mutational alleles and 9 had one mutational allele in the *TYR* gene (Table 1). Forty-three mutational alleles were identified (Table 2 and Figure 1). Fifteen of these have not previously been reported (Table 1): p.C24R, p.W39C, p.W39R, p.R77G, p.C100F, p.C100W, p.M185V, p.H211R, p.R212T, p.Y235H, p.W236R, p.D249G, c.1146C>G (p.N382K), p.A481E, and p.G506X. In addition to these previously unreported *TYR* mutational alleles, 12 alleles reported in other populations (p.H19R, p.G41R, p.R77W, p.R299C, c.1037-7T>A+-10delTT, p.H390D, p.R402G, p.R402X, p.H404P, p.G419R, p.R422Q, and c.1314delCTTT) are reported here in Chinese patients for the first time. Different alleles lead to different changes at the same residue, such as p.C24R/Y, p.R77G/Q/W, and p.C100F/W. Similarly, different alleles may result in the same missense mutation; for example, both c.1146C>A (Tripathi *et al.*, 1992) and c.1146C>G (this study) lead to the same N382K mutation. The c.1037-10delTT allele cosegregates with c.1037-7T>A (Spritz, 1993) in all five of our patients with these alleles. It is worth noting that the previously reported mutational allele c.1217C>T (p.P406L), as shown in patient 18 (Table 1), was identified as an allele from a pseudogene, *TYRL* (Chaki *et al.*, 2005b). Interestingly, c.575C>A (p.S192Y), as found in patient 77 (Table 1), has been reported as a single-nucleotide polymorphism (SNP), rs1042602, in several populations, including Caucasians, Africans, Japanese, and South Asians. However, in the Chinese Han population, the frequency of allele C is 1.00 in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs1042602). Similarly, we did not find the A allele in any of the 100 unaffected subjects from the Han population. The C allele is associated with darker skin (Stokowski *et al.*, 2007). It is uncertain whether the c.575C>A allele contributes to the development of OCA symptoms in a double heterozygous state with other mutational alleles in the Han population.

Among these *TYR* alleles, p.R299H and c.929insC account for 15.4% (26 of 169) and 14.8% (25 of 169), respectively. p.R278X ranks third, with 11.8% (20 of 169). Other alleles with higher frequencies are c.232insGGG (5.9%, 10 of 169), W400L (5.9%, 10 of 169), R116X (5.3%, 9 of 169), C24Y (4.1%, 7 of 169), C55Y (4.1%, 7 of 169), R77Q (4.1%, 7 of 169), and c.1037-7T>A+-10delTT (3.0%, 5 of 169). The above 10 alleles account for 74.6% (126 of 169) of the mutational *TYR* alleles in the Chinese OCA patients in our study. The remaining 33 alleles were found in one or two copies in these patients (Table 2).

Allelic frequencies of *OCA2*, *SLC45A2*, and *HPS1* in Chinese OCA patients

Of the 13 OCA2 patients, 7 had two mutational alleles and 6 had one mutational allele in the *OCA2* gene. Of the 16 mutational alleles, 2 were known mutations (p.P198L and p.L727P), and the remaining 14 were previously unreported mutational alleles (c.168delC, c.980insT, c.860_883del24 bp, c.2351_2376del26 bp, p.R136X, p.K155N, p.R243C, p.Q321P, p.C430X, p.T450K, p.R455G, p.R555C, p.A776D, and p.G782R) (Table 1). The most frequent allele in this screen was p.R455G, which accounts for 15% (3 of 20) of the mutational *OCA2* alleles.

Of the 16 OCA4 patients, 11 had two mutational alleles and 5 had one mutational allele in the *SLC45A2* gene. Of 27 mutational alleles, 11 different alleles were detected (Table 1). All but two—p.D157N and p.D160H (Inagaki *et al.*, 2004; Li *et al.*, 2008)—are previously unreported mutations: c.-5_5delTGGCCATGGG, c.463delG, p.G110R, p.L151P, p.H233Q, p.Y266X, p.G349R, p.E368K, and p.P419L. The most common allele was p.D160H, with an allelic frequency of 55.6% (15 of 27). The p.D160H heterozygotes or homozygotes were clinically diagnosed as either OCA1A or OCA2 (Table 1) on the basis of hair pigmentation.

Finally, a homozygous c.1932delC mutation in *HPS1* was found in a clinically diagnosed OCA1 patient—the first molecularly identified Hermansky–Pudlak syndrome type 1 (HPS1) patient (Wei *et al.*, 2009). In addition, a heterozygous known mutation, c.965insC, which has been reported in Caucasians (Oh *et al.*, 1998), was found in patient 120, who has clinically diagnosed OCA2 (Table 1). This patient was a 5-year-old girl with brown hair, white skin, dark brown irises, remarkable nystagmus, and photophobia. She had no bruises in her lower legs, and all coagulation tests, including activated partial thromboplastin time, prothrombin and thrombin times, and fibrinogen, were within normal ranges.

Table 1. Gene mutations in 127 Chinese OCA patients

Patient no.	Sex	Clinical diagnosis	Molecular diagnosis	Mutation 1	Mutation 2
OCA1/TYR					
1	M	OCA1B	OCA1A	c.832C>T(p.R278X)	c.832C>T(p.R278X)
2	M	OCA1B	OCA1B	c.117G>T(p.W39C)*	c.164G>A(p.C55Y)
3	M	OCA1A	OCA1A	c.232insGGG	c.1199G>T(p.W400L)
4	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.70T>C(p.C24R)*
5	F	OCA1A	OCA1A	c.832C>T(p.R278X)	c.1211A>C(p.H404P)
6	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.1199G>T(p.W400L)
7	M	OCA1B	OCA1A	c.232insGGG	c.1147G>A(p.D383N)
8	F	OCA1B	OCA1B	c.232insGGG	c.1204C>G(p.R402G)
9	F	OCA1A	OCA1A	c.929insC	c.832C>T(p.R278X)
10	F	OCA1A	OCA1A	c.230G>A(p.R77Q)	c.929insC
11	M	OCA1A	OCA1A	c.346C>T(p.R116X)	c.832C>T(p.R278X)
12	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.896G>A(p.R299H)
13	M	OCA1A	OCA1A	c.832C>T(p.R278X)	c.1211A>C(p.H404P)
14	M	OCA1A	OCA1A	c.929insC	c.895C>T(p.R299C)
15	F	OCA1A	OCA1A	c.346C>T(p.R116X)	c.1425G>A(p.W475X)
16	M	OCA1A	OCA1A	c.346C>T(p.R116X)	c.346C>T(p.R116X)
17	F	OCA1A	OCA1A	c.706T>C(p.W236R)*	c.832C>T(p.R278X)
18	F	OCA1A	OCA1B	c.896G>A(p.R299H)	c.1217C>A(p.P406L)@
19	M	OCA1A	OCA1A	c.230G>A(p.R77Q)	c.929insC
20	F	OCA1A	OCA1A	c.117G>T(p.W39C)	c.896G>A(p.R299H)
21	F	OCA1A	OCA1A	c.232insGGG	c.1199G>T(p.W400L)
22	F	OCA1A	OCA1A	c.56A>G(p.H19R)	c.896G>A(p.R299H)
23	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.896G>A(p.R299H)
24	M	OCA1A	OCA1A	c.346C>T(p.R116X)	c.929insC
25	F	OCA1A	OCA1A	c.232insGGG	c.896G>A(p.R299H)
26	M	OCA2	OCA1B	c.164G>A(p.C55Y)	c.553A>G(p.M185V)*
27	F	OCA1B	OCA1A	c.758G>A(p.G253E)	c.929insC
28	M	OCA2	OCA1A	c.832C>T(p.R278X)	c.929insC
29	M	OCA1A	OCA1A	c.929insC	c.1146C>G(p.N382K)*
30	F	OCA2	OCA1B	c.229C>G(p.R77G)*	c.1146C>G(p.N382K)
31	F	OCA1A	OCA1A	c.703T>C(p.Y235H)*	c.632A>G(p.H211R)*
32	F	OCA2	OCA1B	c.832C>T(p.R278X)	c.1037-7T>A+-10delTT
33	M	OCA1A	OCA1A	c.929insC	c.1199G>T(p.W400L)
34	M	OCA2	OCA1B	c.896G>A(p.R299H)	c.1037-7T>A+-10delTT
35	M	OCA1A	OCA1A	c.1199G>T(p.W400L)	c.1314delCTTT
36	M	OCA2	OCA1B	c.71G>A(p.C24Y)	c.1199G>T(p.W400L)
37	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.832C>T(p.R278X)
38	F	OCA1A	OCA1A	c.832C>T(p.R278X)	c.1199G>T(p.W400L)
39	M	OCA1A	OCA1A	c.832C>T(p.R278X)	c.929insC
40	F	OCA1A	OCA1A	c.71G>A(p.C24Y)	c.164G>A(p.C55Y)
41	M	OCA1A	OCA1A	c.164G>A(p.C55Y)	c.346C>T(p.R116X)
42	M	OCA1A	OCA1A	c.71G>A(p.C24Y)	c.232insGGG
43	M	OCA1B	OCA1B	c.1348insGG	c.1199G>T(p.W400L)
44	F	OCA1A	OCA1A	c.929insC	c.929insC

Table 1 continued on the following page

Table 1. Continued

Patient no.	Sex	Clinical diagnosis	Molecular diagnosis	Mutation 1	Mutation 2
45	M	OCA1A	OCA1A	c.832C>T(p.R278X)	c.929insC
46	M	OCA1A	OCA1A	c.71G>A(p.C24Y)	c.346C>T(p.R116X)
47	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.929insC
48	M	OCA1B	OCA1B	c.820-3C>G	c.832C>T(p.R278X)
49	M	OCA1A	OCA1A	c.832C>T(p.R278X)	c.895C>A(p.R299S)
50	F	OCA1A	OCA1A	c.71G>A(p.C24Y)	c.230G>A(p.R77Q)
51	M	OCA1A	OCA1A	c.346C>T(p.R116X)	c.346C>T(p.R116X)
52	M	OCA1B	OCA1B	c.425A>T(p.K142M)	c.896G>A(p.R299H)
53	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.896G>A(p.R299H)
54	F	OCA1A	OCA1A	c.929insC	c.896G>A(p.R299H)
55	F	OCA	OCA1B	c.230G>A(p.R77Q)	c.229C>T(p.R77W)
56	F	OCA	OCA1	c.121G>A(p.G41R)	c.299G>T(p.C100F)*
57	F	OCA	OCA1A	c.832C>T(p.R278X)	c.164G>A(p.C55Y)
58	F	OCA	OCA1A	c.929insC	c.232insGGG
59	M	OCA1A	OCA1B	c.1037-7T>A+-10delTT	c.896G>A(p.R299H)
60	F	OCA1A	OCA1A	c.703T>C(p.Y235H)	c.820-3C>G
61	F	OCA	OCA1B	c.929insC	c.1199G>T(p.W400L)
62	F	OCA	OCA1	c.1516C>T(p.G506X)*	c.895C>T(p.R299C)
63	F	OCA1A	OCA1A	c.929insC	c.1168C>G(p.H390D)
64	F	OCA1A	OCA1A	c.230G>A(p.R77Q)	c.71G>A(p.C24Y)
65	F	OCA	OCA1A	c.896G>A(p.R299H)	c.832C>T(p.R278X)
66	M	OCA1A	OCA1A	c.164G>A(p.C55Y)	c.635G>C(p.R212T)*
67	F	OCA	OCA1A	c.896G>A(p.R299H)	c.929insC
68	M	OCA1A	OCA1A	c.300T>G(p.C100W)*	c.896G>A(p.R299H)
69	F	OCA	OCA1	c.929insC	c.1348insGG
70	F	OCA	OCA1A	c.232insGGG	c.832C>T(p.R278X)
71	F	OCA	OCA1A	c.896G>A(p.R299H)	c.896G>A(p.R299H)
72	F	OCA2	OCA1B	c.1265G>A(p.R422Q)	c.929insC
73	F	OCA1A	OCA1A	c.832C>T(p.R278X)	c.896G>A(p.R299H)
74	M	OCA2	OCA1B	c.1255G>A(p.G419R)	c.1037-7T>A+-10delTT
75	M	OCA1A	OCA1A	c.896G>A(p.R299H)	c.230G>A(p.R77Q)
76	M	OCA2	OCA1B	c.1265G>A(p.R422Q)	c.115T>C(p.W39R)*
77	F	OCA2	OCA1B	c.929insC	c.575C>A(p.S192Y)#
78	F	OCA1B	OCA1A	c.929insC	c.1199G>T(p.W400L)
79	F	OCA1A	OCA1A	c.232insGGG	c.1204C>T(p.R402X)
80	M	OCA1A	OCA1A	c.746A>G(p.D249G)*	c.758G>A(p.G253E)
81	F	OCA1A	OCA1A	c.896G>A(p.R299H)	c.929insC
82	M	OCA1A	OCA1A	c.929insC	c.832C>T(p.R278X)
83	F	OCA	OCA1B	c.895C>A(p.R299S)	—
84	M	OCA1A	OCA1	c.230G>A(p.R77Q)	—
85	M	OCA2	OCA1B	c.1037-7T>A+-10delTT	—
86	M	OCA1A	OCA1	c.232insGGG	—
87	F	OCA1A	OCA1	c.71G>A(p.C24Y)	—
88	F	OCA1B	OCA1	c.1442C>A(p.A481E)*	—
89	F	OCA	OCA1	c.164G>A(p.C55Y)	—

Table 1 continued on the following page

Table 1. Continued

Patient no.	Sex	Clinical diagnosis	Molecular diagnosis	Mutation 1	Mutation 2
OCA2/P					
90	M	OCA2	OCA2	c.2327C>A(p.A776D)*	c.465G>T(p.K155N)*
91	F	OCA2	OCA2	c.2344G>A(p.G782R)*	c.980insT*
92	F	OCA2	OCA2	c.1349C>A(p.T450K)*	c.2180T>C(p.L727P)
93	F	OCA2	OCA2	c.168delC*	c.727C>T(p.R243C)*
94	F	OCA2	OCA2	c.593C>T(p.P198L)	c.593C>T(p.P198L)
95	M	OCA1A	OCA2	c.860_883del24 bp*	c.2351_2376del26 bp*
96	M	OCA1A	OCA2	c.1363A>G(p.R455G)*	c.1290T>A(p.C430X)*
97	M	OCA	OCA2	c.727C>T(p.R243C)	—
98	M	OCA2	OCA2	c.406C>T(p.R136X)*	—
99	M	OCA2	OCA2	c.1363A>G(p.R455G)	—
100	M	OCA2	OCA2	c.962A>C(p.Q321P)*	—
101	M	OCA2	OCA2	c.1663C>T(p.R555C)*	—
102	F	OCA	OCA2	c.1363A>G(p.R455G)	—
OCA4/SLC45A2					
103	M	OCA2	OCA4	c.478G>C(p.D160H)	c.1045G>A(p.G349R)*
104	F	OCA2	OCA4	c.478G>C(p.D160H)	c.478G>C(p.D160H)
105	F	OCA2	OCA4	c.463delG*	c.463delG
106	M	OCA1A	OCA4	c.478G>C(p.D160H)	c.478G>C(p.D160H)
107	F	OCA2	OCA4	c.478G>C(p.D160H)	c.478G>C(p.D160H)
108	F	OCA	OCA4	c.478G>C(p.D160H)	c.478G>C(p.D160H)
109	F	OCA2	OCA4	c.452T>C(p.L151P)*	c.798C>G(p.Y266X)*
110	M	OCA1A	OCA4	c.469G>A(p.D157N)	c.328G>A(p.G110R)*
111	M	OCA1A	OCA4	c.478G>C(p.D160H)	c.1256C>T(p.P419**L**)*
112	M	OCA2	OCA4	c.1102G>A(p.E368K)*	c.478G>C(p.D160H)
113	M	OCA2	OCA4	c.478G>C(p.D160H)	c.478G>C(p.D160H)
114	M	OCA2	OCA4	c.699T>A(p.H233Q)*	—
115	M	OCA2	OCA4	c.478G>C(p.D160H)	—
116	F	OCA2	OCA4	c.-5_5delTGGCCATGGG*	—
117	M	OCA2	OCA4	c.328G>A(p.G110R)	—
118	F	OCA2	OCA4	c.478G>C(p.D160H)	—
HPS1					
119	F	OCA1	HPS1	c.1932delC	c.1932delC
120	F	OCA2	HPS1	c.965insC	—
Unknown					
121	M	OCA	?	—	—
122	M	OCA1	?	—	—
123	M	OCA1A	?	—	—
124	F	OCA2	?	—	—
125	F	OCA2	?	—	—
126	F	OCA	?	—	—
127	M	OCA2	?	—	—

OCA, oculocutaneous albinism.
*Represents previously unknown alleles.
A dash (—) in the mutation column denotes an uncharacterized allelic mutation.
#Indicates a very rare SNP in this population.
@Represents a nonpathological allele amplified from the pseudogene *TYRL*.
? Denotes uncharacterized genotype.

Table 2. Allelic frequencies of the *TYR* gene in 89 Chinese OCA1 patients

Location	Allele	No. of chromosomes	Location	Allele	No. of chromosomes
Exon 1	p.H19R	1	Intron 1	c.820-3C>G	2
	p.C24Y	7	Exon 2	p.R278X	20
	p.C24R	1		p.R299H	26
	p.W39C	2		p.R299C	2
	p.W39R	1		p.R299S	2
	p.G41R	1		c.929insC	25
	p.C55Y	7	Intron 2	c.1037-7T>A+-10delTT	5
	p.R77Q	7	Exon 3	p.N382K	2
	p.R77G	1		p.D383N	1
	p.R77W	1		p.H390D	1
	c.232insGGG	10	Exon 4	p.W400L	10
	p.C100F	1		p.R402G	1
	p.C100W	1		p.R402X	1
	p.R116X	9		p.H404P	2
	p.K142M	1		p.G419R	1
	p.M185V	1		p.R422Q	2
	p.H211R	1		c.1314delCTTT	1
	p.R212T	1		c.1348insGG	2
	p.Y235H	2		p.W475X	1
	p.W236R	1	Exon 5	p.A481E	1
	p.D249G	1		p.G506X	1
	p.G253E	2	Total		169

OCA, oculocutaneous albinism.
Of the 89 OCA1 patients, two mutational alleles were identified in 80, and one mutational allele was identified in 9.

Her platelet count was also within the normal range, but the result of an ADP (adenosine diphosphate)-induced platelet aggregation test was 73% (normal range 20–60%).

Hotspots of *TYR* in Chinese OCA patients

In OCA1, 28 of 43 mutational alleles are located on exons 1 and 2 of the *TYR* gene, accounting for 81.1% (137 of 169) of the total mutational alleles. In particular, four alleles accounting for nearly half of the *TYR* mutations (45.6%, 77 of 169) are located on exon 2 and close to the splicing acceptor of intron 1 (Figure 1). This suggests that exons 1 and 2 are the hotspots of *TYR* mutations in the Chinese Han population.

In OCA4, the p.D160H allele accounts for more than half of the mutational *SLC45A2* alleles, suggesting a founder mutational allele in the Chinese Han population, which is close to another founder allele (p.D157N) among Japanese and Koreans (Inagaki *et al.*, 2005). In *OCA2*, the current reported mutations are sparsely distributed in the entire gene, without apparent mutation hotspots in the Chinese Han population. In the *HPS1* gene, a frameshift hotspot at codons 321–322 is apparent in non–Puerto Rican individuals (Oh *et al.*, 1998). This study is the first to report the c.965insC mutation at this site in a Chinese HPS1 patient.

DISCUSSION

The distribution of disease genes and mutational alleles of a specific gene may vary in different populations. OCA shows this variation within populations. The prevalences of *TYR*, *OCA2*, *SLC45A2*, and *HPS1* are 70.1, 10.2, 12.6, and 1.6%, respectively, in the Chinese population, which are different from those in other populations such as Japanese (Suzuki and Tomita, 2008), non-Hispanic Caucasians (Hutton and Spritz, 2008a), and Danes (Grønskov *et al.*, 2009). However, *TYR* is the most common OCA gene in these four populations, whereas OCA2 is the most frequent form in African and African-American OCA patients (King *et al.*, 2001). Similar to non-Hispanic Caucasians (Hutton and Spritz, 2008a), no mutation in *TYRP1* was found in our OCA repository. However, 10% of Japanese OCA patients have mutations in *HPS1* (Suzuki and Tomita, 2008), but a lower frequency of HPS1 (1.6%) in Chinese OCA patients was reported here, which is attributable to the population-specific spectrum. When molecular testing is conducted in a more comprehensive manner and in more OCA patients, some rare type of OCA or HPS would be expected in Chinese patients. Nevertheless, unidentified OCA patients will provide an important resource for screening potential, previously unreported OCA genes in this repository.

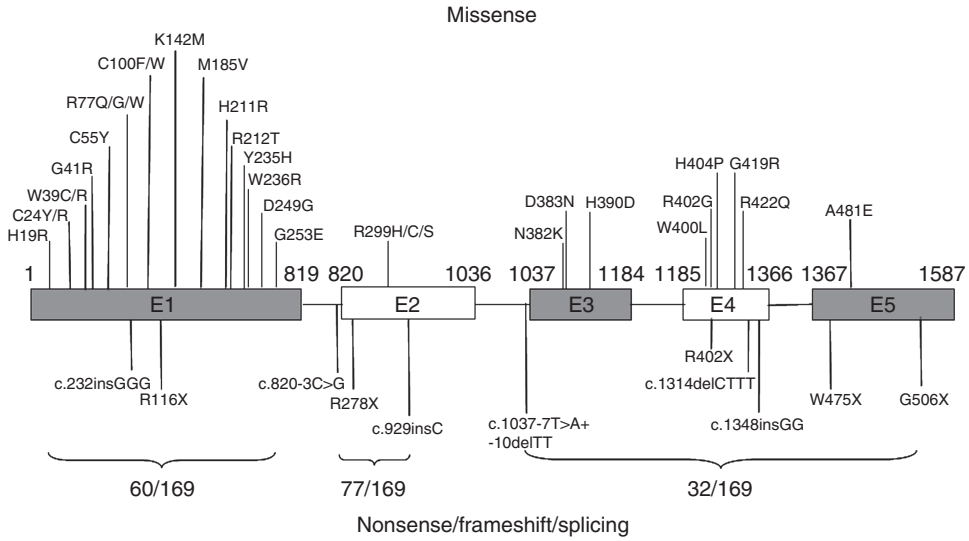

Figure 1. Distribution of mutational alleles of the *TYR* gene in the Chinese Han population. Boxes represent five different exons as indicated, and solid lines connecting these boxes represent the introns of the *TYR* gene. The numbers above the boxes indicate the positions of the *TYR* complementary DNA at the start–stop sites and exon–intron boundaries. Vertical lines represent the locations of the mutational alleles of missense (above the boxes) or nonsense/frameshift/splicing (below the boxes) mutations. The numbers under the brackets denote the total allelic frequencies of the alleles included.

The origin of the mutational alleles of these OCA genes may provide useful information for evolutionary studies of the populations. Interestingly, the p.R299H mutation of *TYR* ranks as the most frequent allele in Chinese (Table 2), Caucasians (Tripathi *et al.*, 1992), Koreans (Park *et al.*, 1997), and Christian Arabs (Zahed *et al.*, 2005), suggesting that it is a common mutational allele in human OCA1. Both the c.929insC and p.R278X alleles in *TYR* have been reported in some Asian populations (Tripathi *et al.*, 1993; Park *et al.*, 1997; Tomita, 2000; Chaki *et al.*, 2005a). Several mutations of *TYR* with higher allelic frequencies, such as p.W400L (Lin *et al.*, 2006), c.232insGGG (Tsai *et al.*, 1999), and p.C24Y (Wang *et al.*, 2009a), have to date been reported only in Chinese OCA patients. Another mutation, p.D160H, is the most common allele of *SLC45A2* in Chinese OCA4 patients.

The mutational spectrum of each OCA gene was found to vary in different populations when a comprehensive analysis was conducted in a larger number of cases. This is evident in Japanese (Suzuki and Tomita, 2008), non-Hispanic Caucasians (Hutton and Spritz, 2008a), and Danes (Grønskov *et al.*, 2009). In Caucasians, *TYR* mutations are clustered in exons 1 and 4 (King *et al.*, 1991; Tripathi *et al.*, 1992). In this study, we found that exons 1 and 2 are the mutational hotspots of the *TYR* gene in the Chinese Han population. These mutational hotspots will be used when designing diagnostic methods in this population.

We did not find the second mutation in 19 patients, after sequencing all the exons and the adjacent exon–intron boundaries of the genes containing the first mutational allele. The percentage of unidentified alleles is similar to that observed in other studies (King *et al.*, 2003; Rooryck *et al.*, 2008; Hutton and Spritz, 2008a; Grønskov *et al.*, 2009). Those ''uncharacterized mutations'' have been suggested to be located upstream of the gene within locus control-like regions, or, alternatively, the mutation may be present at a second locus (digenic scenario) (Passmore *et al.*, 1999; King *et al.*, 2003; Ray *et al.*, 2007; Hutton and Spritz, 2008b). The uncharacterized mutations from the missing gross deletions or insertions were possibly due to amplification of the existing wild-type allele in a regular genomic PCR. In our study, although attempts to screen large deletional mutations on *TYR* or *SLC45A2* were made, no deletions were found (data not shown). Other possibilities of uncharacterized mutations include mutations in unsequenced intronic regions that result in functional changes of proteins.

On the basis of the spectrum of mutational genes and common alleles, we recommend screening for mutations in *TYR*, *SLC45A2*, *OCA2*, and *HPS1* sequentially for genetic diagnosis of OCA in the Chinese Han population and prioritizing the sequencing of hotspots in exons 1 and 2 of *TYR* and in exon 2 of *SLC45A2*. In fact, using our optimized screening strategy, we have reached a much higher success rate (94.5%) in molecular diagnosis of OCA. In addition, we have identified the missed second mutational allele in three patients reported previously (Wang *et al.*, 2009a) in this extensive screening.

In summary, we have described the prevalence of various OCA-causative genes in a Chinese Han population. The mutational spectrum of each OCA gene is different from that found in other populations, and possible mutational hotspots have been described in the *TYR* gene in this population. The genotype–phenotype correlations suggest that molecular diagnosis is more accurate in the differentiation of OCA subtypes. These findings will be useful for gene diagnosis and genetic counseling of OCA in China.

MATERIALS AND METHODS

Study subjects

We recruited 127 unrelated OCA patients and 100 unaffected subjects from the Chinese Han population for a phase II study

(Table 1). The patients in the Chinese Albinism Registry were from more than 20 provinces of China (He and Li, 2007). Only two OCA patients (nos. 44 and 105) had a family history of consanguinity. The 14 OCA1 patients who were described previously (Wang *et al.*, 2009a) were included in this study for extensive molecular analysis and for the calculation of spectral distribution. Typically, among clinically diagnosed OCA1 patients, OCA1A presents with white hair that does not change with age and OCA1B is characterized by white hair at birth that later becomes light yellow or darker (Oetting *et al.*, 2003). OCA2 is characterized by yellow, brown, or golden hair at birth that may darken at a later age. Owing to unclear onset history or too young an age, some patients were not clinically differentially diagnosed; they are listed as OCA or OCA1 in the ''Clinical diagnosis'' column of Table 1. In all 127 OCA patients, white skin, blue or brown irises, and mild to severe nystagmus were observed. This study was approved by the internal review board of the bioethics committees of the Institute of Genetics and Development Biology, Chinese Academy of Sciences, and Xuanwu Hospital, Capital Medical University, and Union Hospital, Peking Union College. The study was conducted according to the Declaration of Helsinki Principles. Written informed consent was obtained and 8 ml peripheral blood samples were collected from all subjects participating in this study.

Genomic amplifications from whole-blood samples

Total genomic DNA was extracted from blood samples using the routine proteinase K/SDS method. Standard PCR amplification procedures were used with an annealing temperature of 58–59 °C for all primers. Primer sequences are available on request. The amplifications cover all the exons and exon–intron boundaries of the five OCA genes: *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, and *HPS1*.

Optimized strategy for mutational screening

Single-strand conformation polymorphism analysis was used for the first round of mutational screening of *TYR*, *OCA2,* and *SLC45A2*. When shifted bands of the polymorphisms were repeated twice, or when no shifted bands were observed, the PCR products were subjected to direct sequencing. When a potentially previously unreported mutation was considered after a careful check with the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/), the Hermansky–Pudlak Syndrome Database (http://liweilab.genetics.ac.cn/HPSD/), and the SNP database (http://www.ncbi.nlm.nih.gov/SNP/), denaturing high-performance liquid chromatography analysis or direct sequencing was used to test the 100 unaffected subjects to exclude the possibility of polymorphism. The procedures for single-strand conformation polymorphism and denaturing high-performance liquid chromatography were described in more detail for the mutational screening of the *GPR143* gene (Wang *et al.*, 2009b). For direct sequencing, all purified PCR products were sequenced using the ABI PRISM 3700 automated sequencer (Applied Biosystems, Foster City, CA).

For all the OCA patients, mutations on the *TYR* gene were first screened. When no mutation in the *TYR* gene was found, the *SLC45A2* and *OCA2* genes were screened sequentially for clinically diagnosed OCA1 patients, and *OCA2* and *SLC45A2* were screened sequentially for clinically diagnosed OCA2 patients. When no point mutation was found on *TYR* and *SLC45A2*, a set of combinational primers was applied to screen deletional mutations. When mutations on *TYR*, *OCA2*, and *SLC45A2* were not found after the above efforts, *TYRP1* and *HPS1* were screened sequentially by direct sequencing. When blood samples were collected from a patient's parents, paternal and maternal origins of the mutational alleles were verified to exclude *de novo* germline mutations and to identify biallelism or triallelism.

Development of a web-based tool for mutation conversion

During mutational screening, when a nucleotide change in cDNA is spotted, the identification of the functional consequence at the protein level is labor intensive, involving a manual check of the mutational position and the genetic codes. Here, we developed a user-friendly Web-based tool, MutConv, for mutation conversion from cDNA to protein by following the guidelines of mutation nomenclature (den Dunnen and Antonarakis, 2000).

The MutConv tool (http://liweilab.genetics.ac.cn/MutConv/) was developed with Perl, running as Apache CGI at the back end and designed with HTML, CSS, and Javascript for the front end. The reference cDNA sequence is put into a box in MutConv. The cDNA mutation is described in a separate box following the rules of mutation description for substitution, insertion, and deletion (http://www.hgvs.org/mutnomen/) (den Dunnen and Antonarakis, 2000). After the program is run, pairwise alignment of the original and mutated cDNA sequences is displayed, followed by pairwise alignment of the original and mutated protein sequences. The mutated base pairs in cDNA or amino acid residues in polypeptide are highlighted. The output includes a description of the mutation at the protein level. MutConv was used to verify all the mutations from cDNA to protein in this study.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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